# Factors Influencing the Activation of Bakers' Yeast

**J. C. Garver, Ina Navarini, and A. M. Swanson**
Departments of Biochemistry and
Dairy and Food Industries
University of Wisconsin, Madison, Wisconsin

IN CONVENTIONAL BREADMAKING, the time required for dough development is more than sufficient for production of the flavor and aroma associated with an active yeast fermentation. The yeast, which starts in a resting state with relatively low activity, also has time to become fully activated and thus is capable of producing sufficient carbon dioxide for the proofing and baking operations. In continuous breadmaking, on the other hand, dough development is accomplished mechanically in a matter of minutes — insufficient time for the development of either an active ferment or the desired flavor and aroma. This has led to the use of a variety of liquid-fermentation processes, usually termed pre-ferments, which precede the dough-mixing stage. A review of some of the factors which govern the activity of bakers' yeast in liquid ferments has been presented by Sykes (1).

In addition to an adequate supply of fermentable sugars, a suitable medium for satisfactory ferment production must include those nutrients essential for anaerobic metabolism, as well as a buffer of sufficient capacity to hold the pH within the optimum range (2) of 4.0 to 5.4. The nutritional requirements of bakers' yeast for anaerobic fermentation have been investigated by Atkin, Schultz, and Frey (3) and for aerobic growth by Olson and Johnson (4). The importance of pH in controlling both gas production and dough quality has been long recognized. The addition of nonfat dry milk (NFDM) to liquid ferments has been found to give good pH regulation and to stablize the ferment so that it can be stored up to 72 hr.[1] An alternative method of pH control is the addition of inorganic compounds such as calcium carbonate to neutralize the organic acids formed during fermentation. Another method, which has been employed in some of this work, is the use of automatic pH control (addition of NaOH) to maintain a constant pH throughout the entire fermentation. This procedure has the advantage, for experimental purposes, of making it possible to separate the buffer effect from the nutritional or chemical action of the various brew components.

The rapid production of a high-activity ferment suitable for continuous breadmaking obviously requires more careful control of such factors as nutrient level, pH, and temperature than is the case for pre-ferments used in conventional bread making. The marked inhibitory effects of sodium chloride and sucrose (at the levels frequently employed), as well as of ethanol, are also important factors in determining the time required for ferment production. Minimization of sugar consumption is another important economic factor. Consideration of these points and of the results to be presented led to the development of a process for continuous production of a liquid ferment suitable for either conventional or continuous breadmaking. Preliminary results with a 20-liter continuous apparatus have demonstrated the feasibility of this method.

## Methods and Apparatus

Three types of fermentation apparatus were employed. The preliminary nutritional work was carried out in a 2-liter round-bottomed flask using 1 liter of culture. The flask was sparged continuously with nitrogen, and the $CO_2$ content of the effluent gas was recorded with a Beckman infrared $CO_2$ analyzer. Subsequent work showed that this method of

Table I. Composition of Batch and Continuous Ferments

| *Component* | *Weight/1,200 g. Flour* *Ferment* | *Added to Dough* | *Total*[a] |
|---|---|---|---|
| | | | % |
| Water | | | |
| Batch | 384 | 414 | 66.5 |
| Cont. | 510 | 288 | |
| NFDM | 0–72 | 0 | 0–6.0 |
| Sucrose | | | |
| Batch | 48 | 60 | 9.0 |
| Cont. | 62.4 | 42.9[b] | 8.75 |
| NaCl | 12–20 | 7–15 | 2.25 |
| Yeast | 27 | 9–15 | 3.0–3.5 |
| Yeast food | | | 1.0 |
| Emulsifier | 1.0 | 2.0 | 0.27 |

Plus lard, malt, and amylase added at dough stage.

[a] Based on flour.
[b] Average residual sucrose in continuous ferment was 15 g./1,200 g. flour.

Table II. Consumption of Base in Controlled-pH Ferments

| *Time* | *pH* 3.5 | 4.0 | 5.1 | 6.1 | 6.5 | 7.0 |
|---|---|---|---|---|---|---|
| | *Total NaOH Added* | | | | | |
| *hr.* | *meq.* | *meq.* | *meq.* | *meq.* | *meq.* | *meq.* |
| 0.5 | 4.5 | 0* | 6.2 | 41 | 56 | 96 |
| 1.0 | 6.3 | 2.1 | 16.8 | 58 | 100 | 158 |
| 2.0 | 6.3 | 5.8 | 26.4 | 76 | 121 | 206 |
| 3.0 | 6.3 | 6.1 | 32.6 | 83 | 134 | 214 |
| 4.0 | 6.3 | 8.0 | 33.1 | 85 | 145 | 217 |
| 5.0 | 6.3 | 8.0 | 33.1 | 85 | 145 | 218 |

[a] Initial pH was 0.11 above control point; hence, no base was called for during the first 30 min.

[1] Choi, R. P. Paper presented at 39th AACC Annual Meeting (1954).

Presented at the 51st Annual Meeting, New York, N.Y., April 1966.
Published with approval of the Director of the Wisconsin Agricultural Experiment Station. Work performed under Contract No. 12-14-100-6877(73), U.S. Dept. of Agriculture, and Project No. 1236, American Dry Milk Institute.

measuring $CO_2$ production rates gave results similar to those for conventional unsparged systems. These runs all were made with dilute yeast suspensions containing 1.14 g./liter dry weight of yeast. As was also the case in all of the latter work, the approximate weight of yeast required was suspended in water and the correct volume of yeast suspension for each run determined by absorbance measurements.

The aparatus used for batch production of conventional liquid ferments consisted of four 2-liter glass fermenters with variable-speed agitators. These fermentation vessels were operated in a constant-temperature bath, and each unit was connected to a separate recording gasometer which gave a continuous record of the rate of $CO_2$ production. The composition of the ferments used in this phase of the work is given in Table I. For the controlled-pH ferments, a pair of modified Leeds & Northrup miniature pH electrodes was installed in each fermenter and connected to a Leeds & Northrup pH meter coupled to a relay. The relay operated a motor-driven syringe by means of which 1*N* to 5*N* sodium hydroxide was added to maintain the pH at the desired control point.

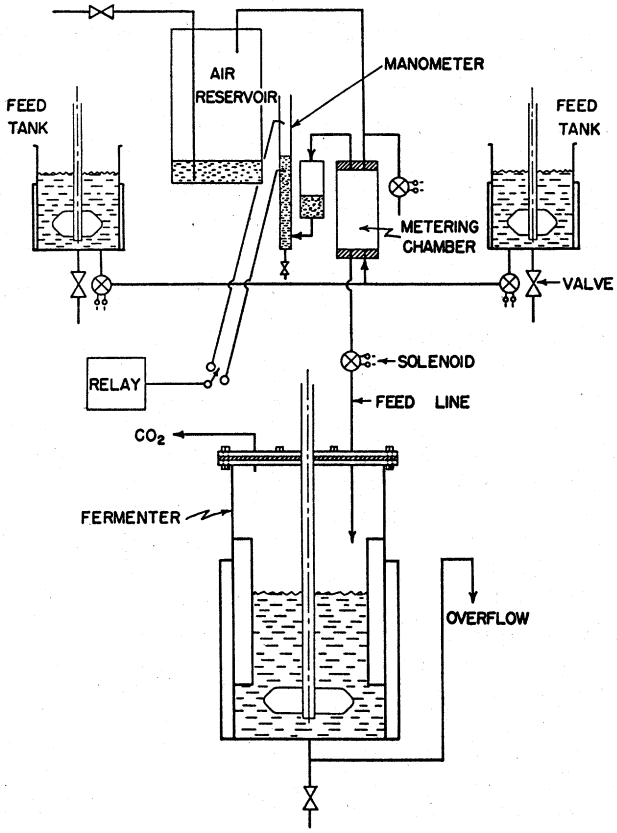


Fig. 1. 30-liter continuous fermenter.

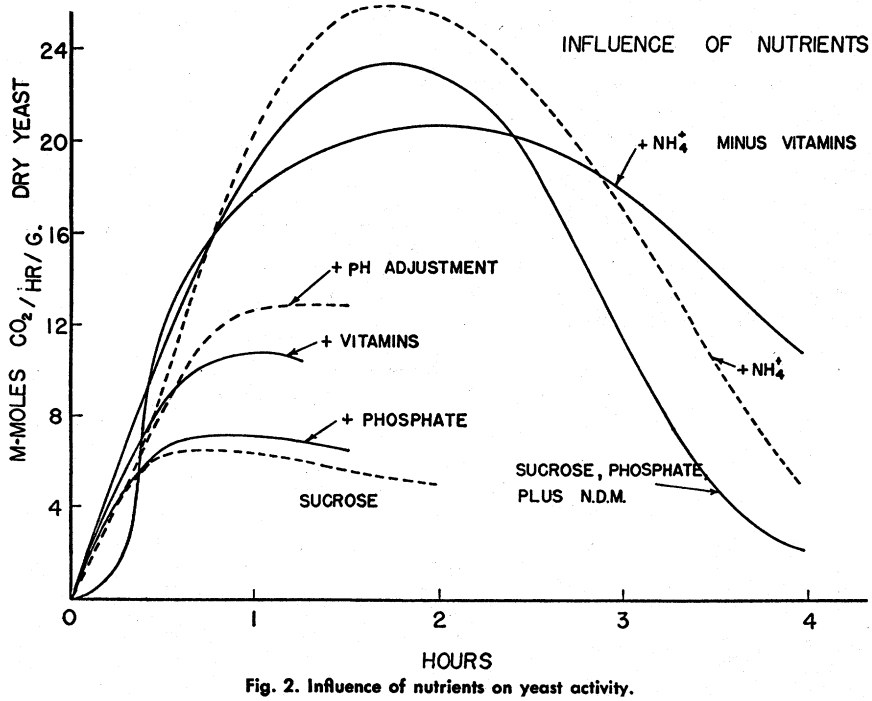


Fig. 2. Influence of nutrients on yeast activity.

Figure 1 is a schematic diagram of the continuous-fermentation apparatus. This unit consisted of a 60-liter, jacketed, stainless-steel fermenter, two feed tanks, and a metering device for the addition of yeast suspension (feed tank No. 1) and nutrients (feed tank No. 2). The fermenter was provided with an overflow line, the position of which could be adjusted to give liquid hold-up volumes of 12 to 30 liters. The metering system consisted of four solenoid valves, a metering chamber, and a cycling timer. This made it possible to add fixed volumes of feed (25–150 ml.) alternately from each feed tank according to a fixed cycle (0.5–4 min.). The feed rate could be changed by varying either the capacity of the metering chamber or the cycle time. The hold-up time for the fermenter thus could be varied from 40 min. to 6 hr. The composition of the ferments used with this system is given in Table I. Since it was not possible to measure carbon dioxide production rates directly with this fermenter, the fermentations were followed by means of periodic sucrose analyses by the Shaffer-Somogyi procedure (5).

Baking tests were carried out in form-loaf batches; the amount of ferment was equivalent to 1,200 g. of flour. The baking procedures are described and more detailed results are presented in another article (6). The scoring of the bread produced from continuous ferments was done by an experienced baker according to the procedures of the American Dry Milk Institute.[2]

## Results

The data obtained on the influence of various nutrients on yeast activity are summarized in Fig. 2. Fermentations with either sucrose alone or sucrose plus inorganic phosphate leveled off after 30 min. at a low rate of

[2] Swortfiguer, M. J., personal communication.

$CO_2$ production. The further addition of seven growth factors (inositol, thiamine, riboflavin, nicotinamide, pantothenic acid, pyridoxine, and biotin) substantially increased the maximum activity. Activity was increased further by adjustment of the initial pH of the ferment from 3.6 to 6.7 by addition of sodium hydroxide. This procedure was followed in all subsequent runs of this type to avoid the inhibitory effects of low pH. The maximum rate of 24 millimoles $CO_2$/hr./g. dry yeast was obtained with a medium containing sucrose, phosphate, ammonium sulfate, and the seven vitamins listed above. Omission of the vitamins reduced the peak activity by about 20%, although the activity still was appreciably higher than for any of the systems which did not contain ammonium ions. The activity observed for a sucrose-phosphate-NFDM system, while not quite as high as with the best synthetic medium, demonstrates that nonfat dry milk is an excellent source of the trace nutrients required for maximum yeast activity. Microscopic observations of the yeast cells during these fermentations indicated that maximum activity is associated with the presence of 20–30% buds. Although no significant increase in the dry weight of yeast was observed, these observations suggest that a limited amount of growth is essential for the production of maximum activity.

The majority of the batch ferments produced with the medium listed in Table I contained 6% NFDM based on flour. From the results described above, it was evident that the trace nutrients were present in sufficient amounts and that the yeast activity would be controlled by pH, temperature, and the presence of inhibitory substances. The marked inhibitory effect of sodium chloride on bakers' yeast is shown in Fig. 3. In this case, the lowered rate of $CO_2$ production was carried over to the dough, as shown by the decreased loaf volumes. As was expected, the bread without salt had a flat taste; in all other respects, however, it compared favorably with the bread produced from ferments containing higher salt concentrations. Somewhat similar results also were observed when the initial concentration of sucrose was varied. Sugar concentrations above 2.0% considerably increased the time re-

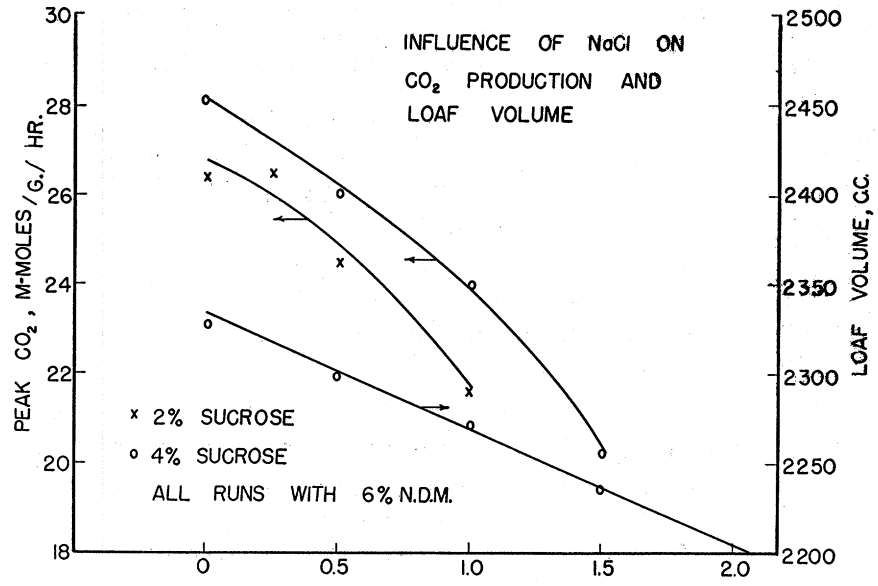


Fig. 3. Influence of NaCl on $CO_2$ production and loaf volume.

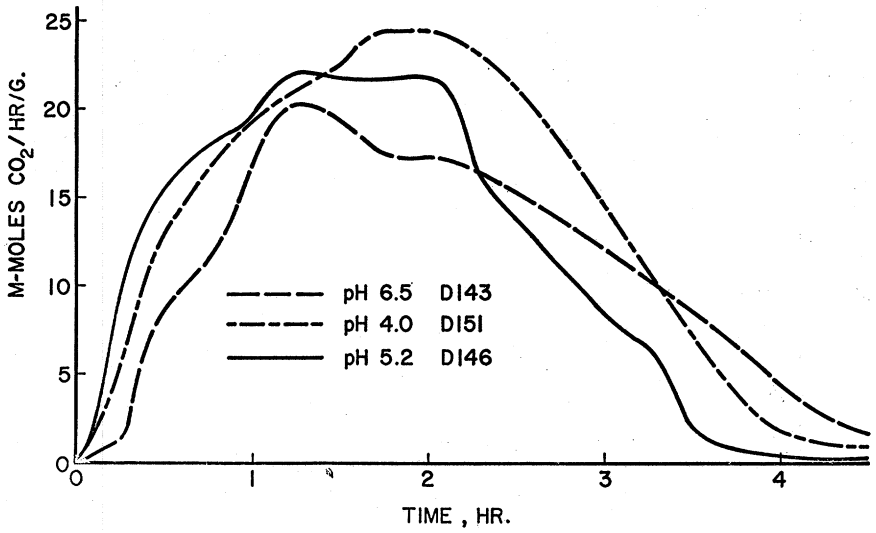


Fig. 4. Controlled-pH ferments.

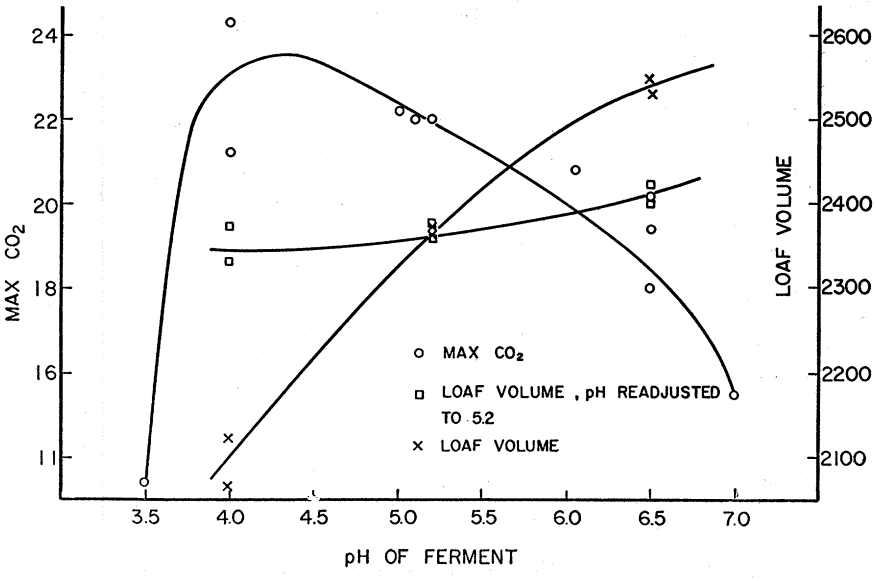


Fig. 5. Results of series of controlled-pH runs.

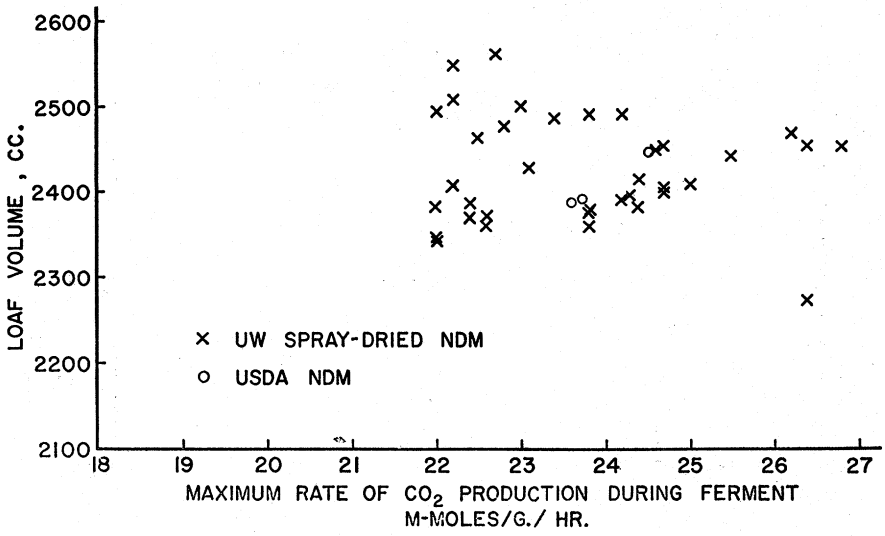


Fig. 6. Comparison of loaf volume with $CO_2$ production during ferment.

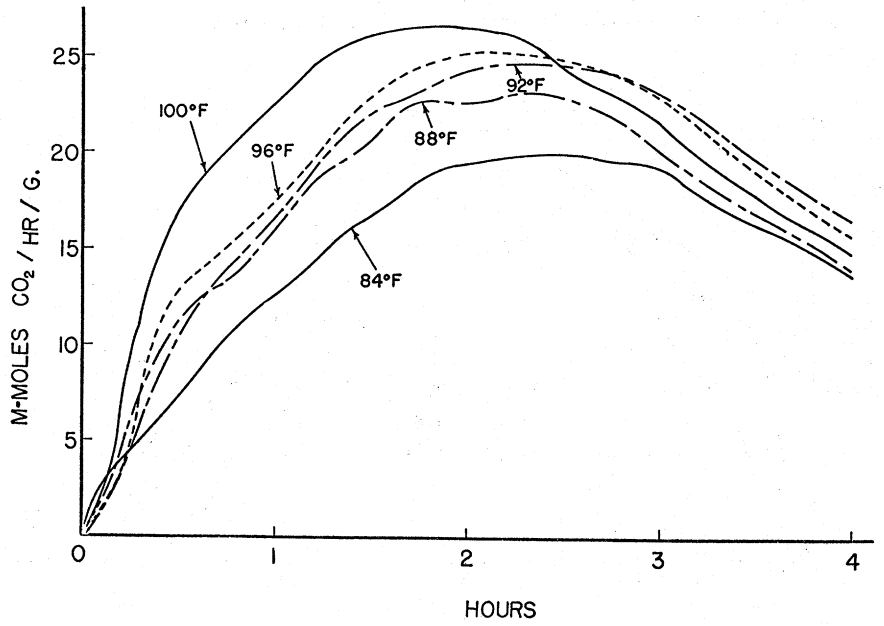


Fig. 7. Influence of temperature on activity of liquid ferment, 84°–100°F.

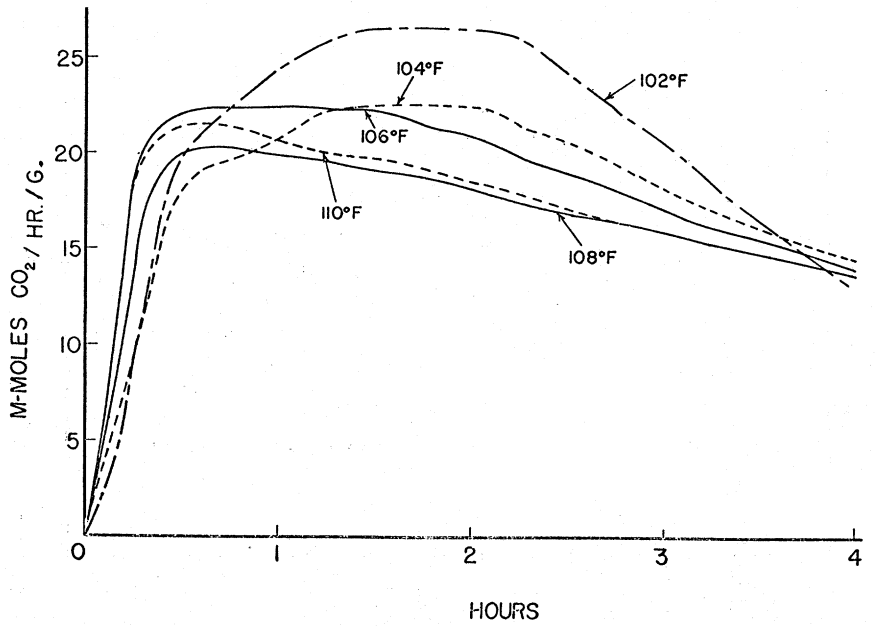


Fig. 8. Influence of temperature on activity of liquid ferment, 102°–110°F.

quired for ferments to reach peak activity.

The pH of 6% NFDM ferments dropped rapidly from initial values of 5.9–6.3 to below 5.5 during the first 30 min. The pH then decreased slowly to a minimum value of 5.1 after 3.5 to 4.0 hr. In the absence of NFDM, the pH dropped rapidly to values below 4.0. To separate the influence of pH from the nutritional or chemical effects of the buffer, a series of controlled-pH runs was made with 6% NFDM ferments. The $CO_2$ production curves for three of these runs are given in Fig. 4, and the results of this series are summarized in Fig. 5. The peak activity of the yeast was observed to increase as the pH was varied from 7.0 to 4.0 and then to drop abruptly between pH 4.0 and 3.5. The influence of ferment pH on loaf volume, however, was exactly the opposite. This indicates that pH must influence other factors, such as $CO_2$ retention in the dough, which override its effect on yeast activity. In a parallel series of runs, where the pH of the ferment was adjusted to 5.2 before incorporation in the dough, no effect of fermentation pH on loaf volume was observed.

The consumption of base (NaOH) during these controlled-pH ferments is given in Table II. The rate of acid production during the fermentations increased markedly at pH values above 5.0. Since the formation of products other than carbon dioxide and ethanol thus increases at higher pH, high-pH ferments might possibly be of value for flavor production.

The nature of the heat-treatment given to skim milk prior to drying is known to influence the baking quality of NFDM. To investigate the influence of these factors on the behavior of liquid ferments, a series of 46 ferments (6% NFDM) was made with 20 different samples of NFDM. The heat-treatments used in preparing these milk samples varied from none to 205°F. for 30 min. prior to concentration. In addition, half of the samples were heated at 175°F. for 10 min. after evaporation. Although the maximum rate of $CO_2$ production for these ferments varied from 22 to 27 millimoles/g./hr., no definite trend was observed with either forewarming temperature or heat-treatment of the concentrate. The observed variations are thought to be the result of random variations

in the level of available nutrients from one batch of NFDM to another. A summary of these results is shown in Fig. 6. It is apparent that no definite correlation exists between performance of the ferment, as measured by peak $CO_2$ activity, and baking performance measured by loaf volume.

With the possible exception of pH, the most important factor which influences the activity of a liquid ferment is temperature. The results of a series of 6% NFDM batch ferments at temperatures from 84° to 110°F. are presented in Figs. 7 and 8. The maximum level of yeast activity was reached at temperatures of 100° to 102°F. Of even greater significance is the fact that the initial rate of activation was found to increase rapidly with temperature. For example, at 88°F. a total of 90 min. was required to reach an activity of 20 millimoles $CO_2$/hr./g. whereas at 110° F. only 15 min. was needed to reach the same activity. It would thus appear that temperatures somewhat higher than those commonly used might provide a way to appreciably reduce the time required to produce a satisfactory ferment.

The continuous-culture apparatus shown in Fig. 1 was designed to investigate the feasibility of continuous ferment production. The results of four of the preliminary continuous runs are presented in Fig. 9. The course of these fermentations was followed by means of periodic sucrose assays. In all cases the fermenter was filled with 20 liters of culture (Table I) and operated as a batch fermentation for 0.5–2.0 hr. The feed was then started at a rate which gave a hold-up time of either 1 or 2 hr., and continued until steady state was established as indicated by a constant sucrose level in the effluent. To minimize the time required for the system to reach a steady state, it was necessary to operate the fermenter batchwise until the sucrose concentration fell to the eventual steady-state value. The results of this type of run are shown in Fig. 10, where continuous feeding was begun at 1.75 hr. and steady-state conditions maintained for over 16 hr.

The results of baking tests conducted with continuous ferment are given in Table III. In this case, feed was begun at 1.5 hr. and effluent collected every 30 min. thereafter for the baking tests. The two control runs were made from batch ferment produced by the methods previously described. Proof time with the continuous ferment was 10% less than that required for the controls, and average loaf volume was approximately 50 cc. less. No significant difference in the scores was observed, except for the sample at 2.5 hr. where sugar was inadvertently omitted at the dough stage. Although steady-state conditions had not been reached at 2.0 hr., as shown by the sucrose assay, the results with this sample did not differ from subsequent ones.

**Table III. Baking Tests with Continuous Ferments**

| *Sample* | *Proof Time (to Template)* | *Sucrose[a] in Ferment* | *Loaf Volume* | *Score* |
|---|---|---|---|---|
| *hr.* | *min.* | *g.* | *cc.* | |
| 2.0 | 59 | 20.3 | 2,435 | 91+ |
| 2.5 | 59 | 15.9 | 2,455 | 85 |
| 3.0 | 59 | 16.0 | 2,535 | 92 |
| 3.5 | 59 | 15.1 | 2,485 | 90− |
| 4.0 | 59 | 15.9 | 2,473 | 91− |
| 4.5 | 59 | 15.1 | 2,475 | 89− |
| 5.0 | 59 | 15.5 | 2,423 | 89+ |
| Control 1 | 66 | 0 | 2,507 | 93+ |
| Control 2 | 64 | 0 | 2,480 | 88+ |

[a] Weight per 1,200 g. flour.

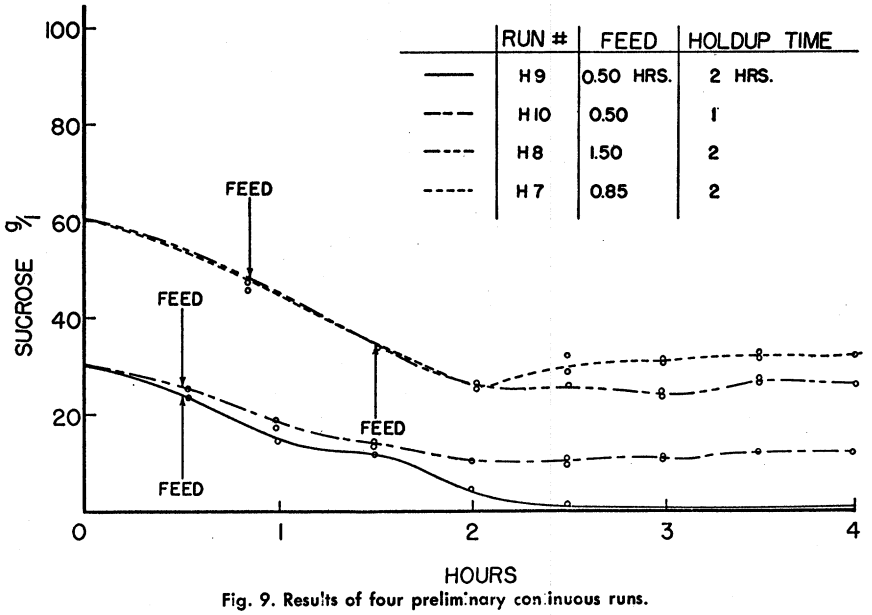


Fig. 9. Results of four preliminary continuous runs.

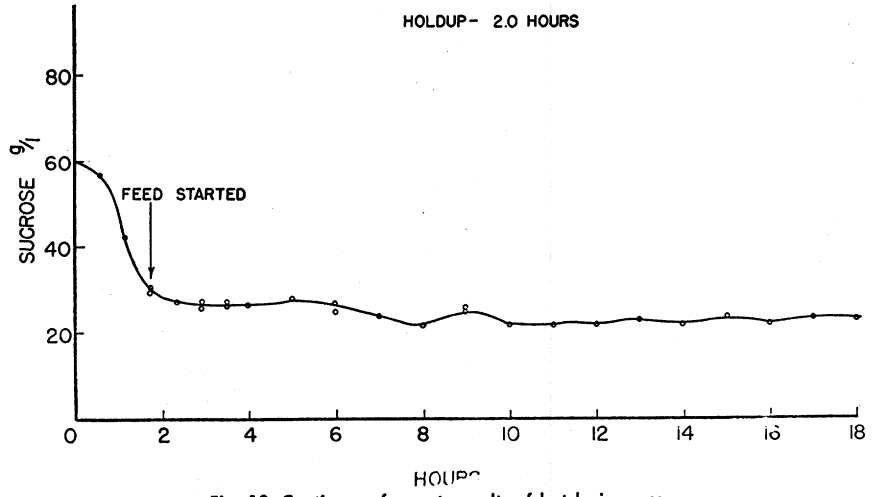


Fig. 10. Continuous ferment; results of batchwise runs.

## Discussion

The results with the continuous ferment system, while by no means conclusive, are sufficiently encouraging to warrant further study. The advantage of a continuous culture system include greater product uniformity, ease of controlling pH and

nutrient levels, and greater output per unit fermenter volume. The disadvantages include greater cost for instrumentation and power and a decrease in flexibility of operation.

The advantages of a continuous fermentation system result from the fact that, once steady-state conditions are reached, the concentrations of all components of the brew remain constant. The activity of the effluent thus remains constant, and the variations inherent in all batch methods are avoided. Since the effluent sugar concentration may be maintained at as low a level as desired, the inhibitory effect of high sugar concentrations can be eliminated. The pH will also remain constant and can be adjusted by controlling both the amount of buffer and the pH of the nutrient feed solution. The advantages of a controlled-pH fermentation are thus obtained without the elaborate instrumentation required for pH control in a batch operation.

In the case of fermentations exceeding 3 hr. in length, little advantage would accrue from continuous operation. As the hold-up time required for production of a satisfactory ferment is decreased, however, the advantage of a continuous system increases. The results obtained at elevated temperatures are significant in this respect, since increasing temperature is the only way of appreciably reducing the hold-up time.

Although processing variables are known to influence the baking quality of nonfat dry milk, no evidence of any systematic influence of heat-treatment on ferment behavior was observed. None of the NFDM ferments exhibited $CO_2$ production rates as high as those observed with synthetic ferments; however, no evidence of any depression in fermentation rates resulting directly from the presence of NFDM was observed. The peak activity increased with increasing NFDM concentration, which is not consistent with the hypothesis that NFDM contains substances which inhibit fermentation. Off-odors in bread — which have been attributed, in some cases, to the enzymatic action of yeast on milk powder — also were not observed.


## Acknowledgment

The authors wish to thank M. J. Swortfiguer for his assistance in the development of the baking procedures used in this work, and Universal Foods, Milwaukee, Wisconsin for supplying the bakers' yeast.


## Literature Cited


1. Sykes, H. G. *Bakers' Dig. 48:* (Aug. 1959).
2. Maselli, J. A. *Bakers' Weekly 168*(6): (1966).
3. Atkin, L., Schultz, A. S., and Frey, C. A. *Cereal Chem. 22:* 321 (1948).
4. Olson, B. H., and Johnson, M. J. *J. Bacteriol. 57:* 235 (1949).
5. Shaffer, P. A., and Somogyi, M. *J. Biol. Chem. 100:* 695 (1933).
6. Swanson, A. M., Seibel, J. K., Sanderson, W. B., and Garver, J. C. *Cereal Sci. Today 11:* 398 (1966).